Age-Related Variations of Caveolins Expression in the Wall of Saphenous Vein Used for Coronary Artery Bypass Grafting. A Preliminary Report

Bartłomiej Perek[1,*], Agnieszka Malińska[2], Danuta Ostalska-Nowicka[3], Maciej Zabel[2], Marek Jemielity[1] and Michał Nowicki[2]

[1]*Department of Cardiac Surgery and Transplantology,* [2]*Department of Histology and Embryology,* [3]*Department of Pediatric Cardiology and Nephrology University of Medical Science, Poznan, Poland*

Abstract: *Background*: Smooth muscle cells (SMCs) in the tunica media of the saphenous vein (SV) used for aortocoronary grafts play a key role in intimal hyperplasia. This process, followed by the development of atherosclerosis, leads to occlusion of the SV grafts months and years after coronary artery bypass grafting (CABG). Caveolins have been suggested to control some of the SMCs responses. The aim of this study was to identify any variations in expression of caveolins (cav-1, cav-2 and cav-3) in the SMCs of the SV transplants in relation to the age of CABG who undergo CABG.

Methods: This study involved 110 (83 males and 27 females) consecutive, isolated and non-emergent CABG patients. They were retrospectively divided into four age subgroups: (A; n=19) ≤50 year-old, (B; n=24) >50 and ≤60 year-old, (C; n=40) >60 and ≤70 year-old, (D; n=27) >70 year-old. Expression of cav-1, cav-2 and cav-3 in the SV wall was evaluated by means of immunohistochemistry.

Results: Expression of cav-1 and cav-3 in the SV wall increased systematically with the age of CABG patients and was statistically significant between group A and D ($p<0.05$). A positive correlation between the age of patients and expression of cav-1 and cav-3 was found ($r=0.450$ and $r=0.463$, respectively). Cav-2 expression did not differ between the examined subgroups.

Conclusion: This study revealed positive correlations between the expressions of cav-1 and cav-3 in the medial venous SMCs and the age of CABG patients. However, further investigations are necessary to show clinical significance of these age-related variations.

Keywords: Saphenous vein, coronary artery bypass grafting, age, caveolins, immunohistochemistry.

INTRODUCTION

An interposition of the saphenous vein (SV) between the aorta and the recipient coronary arteries during coronary artery bypass grafting (CABG) initiates a series of biological events within the venous wall. The formation of neointima serves as the foundation for subsequent progressive occluding graft atheroma [1, 2]. Patency of the grafts is considered a critical determinant of CABG long-term outcomes. Phenotypic transformation of the smooth muscle cells (SMCs) in the tunica media of the SV grafts followed by abnormal proliferation and migration through internal elastic lamina, are key factors in neointima development [3, 4]. SMCs respond to a variety of biologically active molecules released by the injured endothelium, platelets and activated macrophages [5, 6]. A number of studies have demonstrated that cellular SMCs response is mediated by specialized 50 – 100 nm flask-shape plasma membrane invaginations known as caveolae [7, 8]. The major protein caveolar components are caveolins. Currently, three different types have been identified: caveolin-1 (cav-1), caveolin-2 (cav-2), and caveolin-3 (cav-3) [9-11]. Cav-1 has been suggested to inhibit proliferative responses of the SMCs to platelet-derived growth factor (PDGF) [12, 13]. PDGF and basic fibroblast growth factor that are able to induce SMCs stimulation, decreases with age [14]. Notably, elderly individuals constitute a constantly increasing population of patients undergoing CABG [15].

Thus, the aim of this study was to identify any variations in expression of cav-1, cav-2 and cav-3 in the SMCs of the SV transplants in relation to the age of CABG patients.

MATERIAL AND METHODS

Study Group

Institutional Review Board at Poznan University of Medical Sciences approved a research protocol (Approval No.1201/08). An informed written consent from each study participant was obtained. One hundred ten consecutive patients (83 males and 27 females with the age ranging from 46 to 85 years) who underwent

*Address correspondence to this author at the Department of Cardiac Surgery and Transplantology, Poznan University of Medical Sciences in Poznan, Ul. Dluga1/2, 61-848 Poznan, Poland; Tel/Fax: +48 61 8549085;
E-mail: bperek@yahoo.com

E-ISSN: 2311-052X/13 © 2013 Synergy Publishers

Table 1: Preoperative Demographic and Clinical Data

	Group A [n=19]	Group B [n=24]	Group C [n=40]	Group D [n=27]
Age [mean±sd]	48.4 ± 1.3	56.0 ± 1.1	64.5 ± 2.6	74.3 ± 3.8
Gender [M/F]	18/1	19/5	29/11	17/10
BMI	30.6 ± 3.6	29.5 ± 4.5	29.4 ± 4.1	27.5 ± 4.4
Obesity (BMI>30) [n(%)]	10 (53)	12 (50)	16 (40)	7 (26)
Stable angina [n(%)]	17 (89)	19 (79)	34 (85)	22 (81)
Unstable angina [n(%)]	2 (11)	5 (21)	6 (15)	5 (19)
History of infarct [n(%)]	15 (79)	14 (58)	28 (70)	17 (63)
Previous PCI [n(%)]	10 (53)	7 (29)	13 (33)	6 (22)*
Arterial hypertension [n(%)]	14 (74)	19 (79)	31 (78)	15 (56)
Diabetes mellitus [n(%)]	6 (32)	10 (44)	23 (58)	14 (52)
Hyperlipidemia [n(%)]	10 (53)	10 (42)	17 (43)	9 (33)
PVD [n(%)]	5 (26)	7 (29)	7 (18)	10 (37)
Neurological events [n(%)]	0	2 (8)	4 (10)	3 (11)

*p<0.05 group A vs group D; BMI = body mass index; CAD = coronary artery disease; PCI = percutaneous coronary intervention; PVD = peripheral vascular disease.

isolated non-emergent CABG with the use of at least one aortocoronary bypass graft between November 2008 and February 2009 were enrolled in the study. They were retrospectively divided into four subgroups according to the age at the time of surgery: (A; n=19) ≤50 year-old, (B; n=24) >50 and ≤60 year-old, (C; n=40) >60 and ≤70 year-old, (D; n=27) >70 year-old. The most important preoperative demographics and clinical history data are summarized in Table **1**.

Operation Procedure and Sample Collection

All surgeries were performed from median sternotomy. A part of them were carried out on the beating heart (OPCAB, off-pump coronary artery bypass) and the others in the extracorporeal circulation (CCAB, conventional coronary artery bypass). The studied age-matched subgroups did not differ with respect to surgical technique of CABG (OPCAB vs. CCAB), a number and type of the aortocoronary grafts (arterial vs. venous), and the rate of complete revascularization. Some intra-operative data are presented in Table **2**.

SV was harvested using continuous leg incision over its course with the use of "no-touch" technique. The most distal part of the harvested SV segments (at least 1.5 – 2 cm long) were saved for immunohistochemical examinations. These samples were carefully rinsed with 0.9% NaCl at the room temperature, fixed in the freshly prepared Bouin's solution. Then the samples were transferred to the

Table 2: Intraoperative Data in the Subsequent Studied Groups

	Group A [n=19]	Group B [n=24]	Group C [n=40]	Group D [n=27]
OPCAB [n]	7	10	18	10
CCAB [n]	12	14	22	17
Number of grafts/pt	3.0 ± 0.7	2.6 ± 0.6	2.7 ± 0.6	2.4 ± 0.6
Grafts type [distal anastomoses n]				
Arterial (LITA, RITA, RA) [n]	24	23	41	26
Venous [n]	33	39	67	39
Complete revascularization[a][n(%)]	16 (84)	16 (67)	23 (58)	18 (70)

ns; [a]all severely stenotic or occluded coronary arteries were bypassed.
Abbreviations:
CCAB = conventional coronary artery bypass (in the extracorporeal circulation); LITA = left internal thoracic artery; OPCAB = off-pump coronary artery bypass; RA = radial artery; RITA = right internal thoracic artery.

Department of Histology and Embryology, where they were embedded in paraffin and cut into 5-6 μm thin sections on a semi-automatic rotary microtome (Leica RM 2145, Leica Microsystems, Nussloch, Germany).

Immunohistochemical Analysis

The immunohistochemical analysis employed StreptABComplex/HRP method modified by biotinylated tyramine (Dako Catalysed Signal Amplification System, Peroxidase, K1500, Dako Denmark A/S, Glostrup, Denmark) [16]. In marker detection, the chromogen reaction was developed using 0.5% 3-3' diaminobenzidine (Sigma Chemical Co., St. Louis, MO) in TRIS/HCl, pH 7.6 plus 0.3% H_2O_2.

Following monoclonal mouse anti-human antibodies were used: anti-cav-1 (diluted 1:300, NB100-615, Novus Biologicals, Cambridge, GB), anti-cav-2 (diluted 1:100, sc-7942, Santa Cruz Biotechnology, Heidelberg, Germany) and anti-cav-3 (diluted 1:500, NB100-5029, Novus Biologicals).

The intensity of the immunostaining was quantified using the computer software AxioVisionLE for Windows ver.4.8.2 on the images of the SV samples captured with an AxioCam MRc5 digital camera attached to an AxioImager Z.1 light microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany). Tissue expression was evaluated semi-quantitatively employing the following classification, where (0) meant no immunoreactivity; (1+) – up to 25%; (2+) – between 26% and 50% and (3+) – more than 50% of the immunostained tunica media.

All the immunohistochemical analyses were performed blind by two experienced pathologists on coded samples and complied with the principles of positive and negative controls. Median values of 10 representative microscopic fields (double analysis) located evenly around the vessel wall circumference were entered into further calculations and statistical analyses. The negative controls were performed using normal mouse IgG antibodies or phosphate buffered solution. In addition, the serial sections were stained in the subsequent experiments as positive controls to determine the consistency of staining.

Data Management and Statistical Analysis

Initially, all continuous variables were estimated for normality with the Shapiro-Wilk *W* test. When they

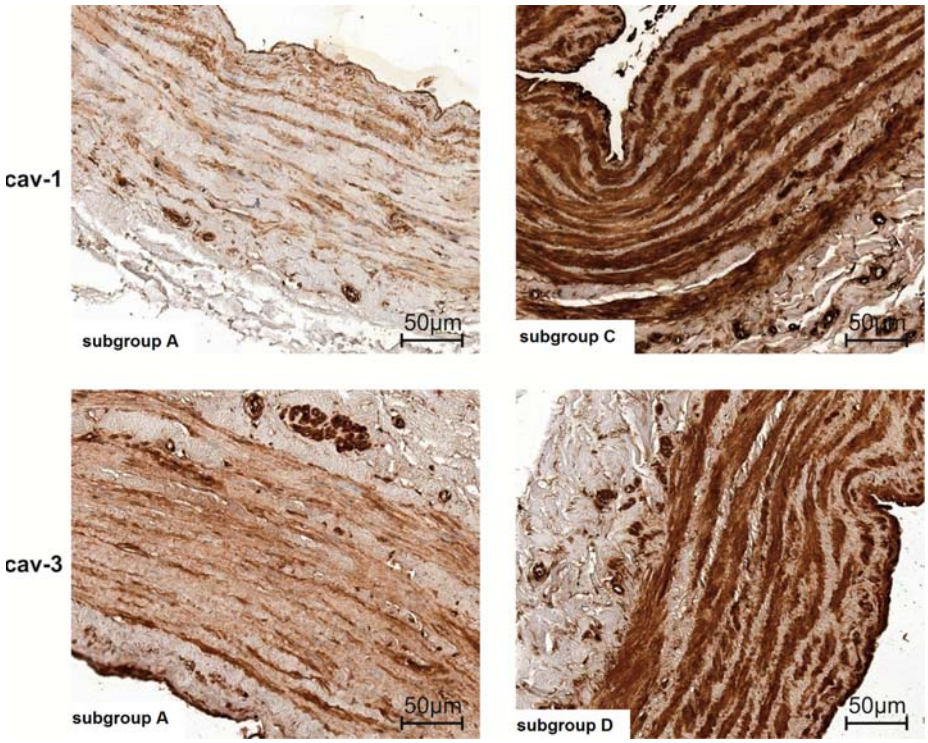

Figure 1: Immunostaining for cav-1 and cav-3 within the tunica media of the SV grafts.
Immunohistochemical analysis of the caveolins' expression revealed less pronounced cav-1 immunostaining in the younger subpopulation (subgroup A; a 47-year old male) (left upper specimen; 1+ expression) than in the older one (subgroup C; 68-year male) (right upper; 3+). Likewise, expression of cav-3 was weaker in the youngest (subgroup A; a 49-year female) (left lower; 1+) than in the oldest CABG patients (subgroup D; a 74-year male) (right lower; 3+).

complied with a normal distribution they were expressed as mean ± standard deviation. The Nominal data (ie. semi-quantitative caveolin expression) were compared with the use of a Kruskal-Wallis test for multiple rang comparisons. Correlations between the patient age and expression of caveolins were tested with the use of a non-parametric R Spearmann correlation test. A *p* value < 0.05 was regarded as statistically significant. Analyses were carried out using Statistica 9.0 for Windows (StatSoft, Inc., Tulsa, OK, USA).

RESULTS

Expression of either cav-1 or cav-3 in the tunica media of the SV segments was detected in the samples obtained from all patients. Cav-1 expression increased systematically with the age of CABG patients (subgroup A < subgroup B < subgroup C < subgroup D; p<0.05) (Figure **1**).

The significant differences in the Kruskal-Wallis test were found between groups A and D (p = 0.015), and between groups A and C (p = 0.007) (Figure **2**). Statistical analysis also confirmed significant positive correlation between the age of CABG patients and cav-1 expression (r=0.450; p<0.001).

Cav-2 expression in the media of the SV did not differ significantly between examined subgroups of age-matched CABG patients. Contrary to the other caveolins, in some SV segments no cav-2 expression

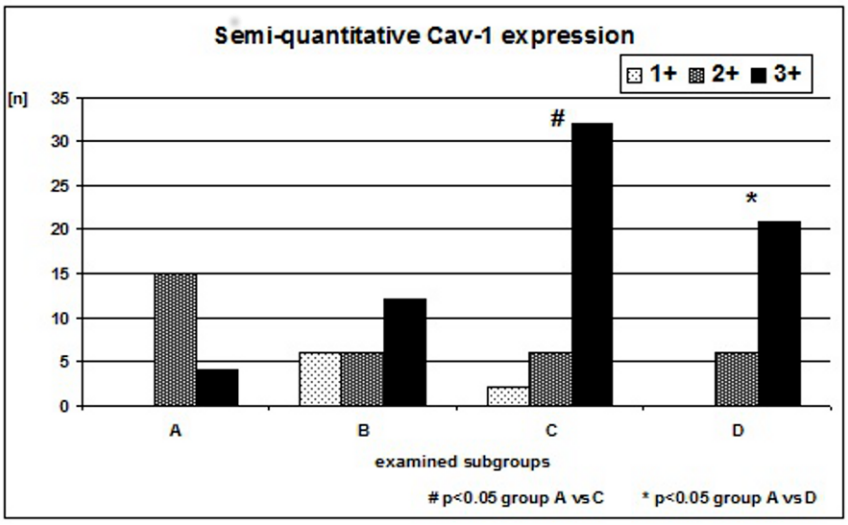

Figure 2: Semi-quantitative expression of cav-1 in the saphenous vein tunica media in particular subgroups.

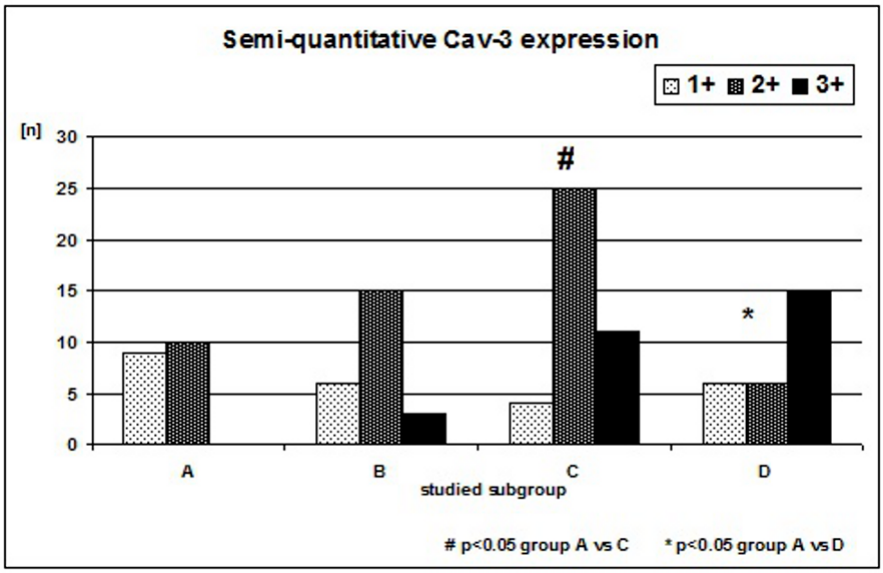

Figure 3: Semi-quantitative expression of cav-3 in the saphenous vein tunica media in particular subgroups.

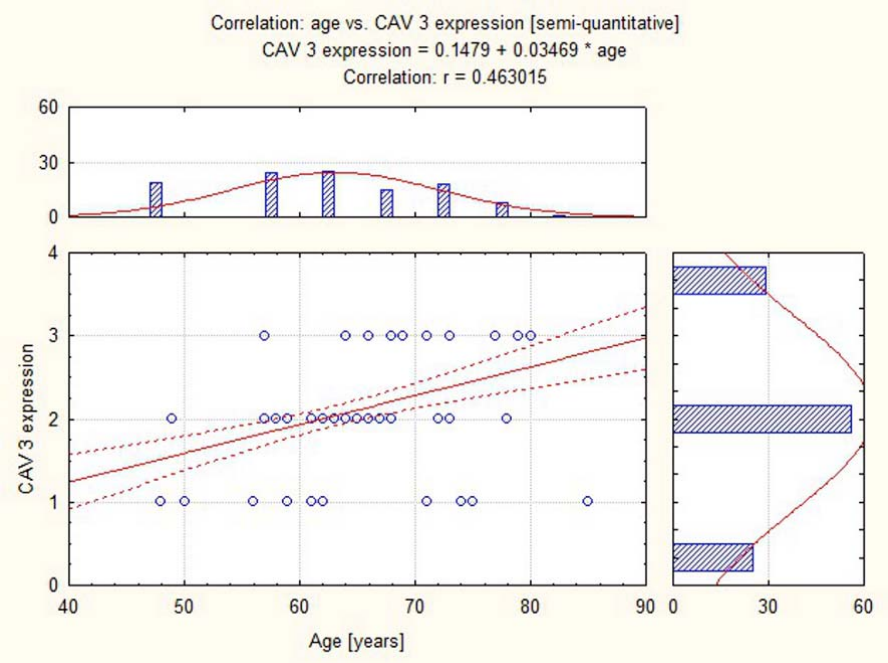

Figure 4: Significant moderate positive correlation between expression of cav-3 and the age of CABG patients.

was found. The rate of cav-2-negative SV segments ranged from 25% (subgroup B) to 47% (subgroup A).

Cav-3 expression in the SV media increased with the age of CABG patients (p=0.0005 in Kruskal-Wallis test) (group A < group B < group C < group D) and differed significantly between groups A and D (p= 0.002), and between groups A and C (p= 0.015) (Figure **3**). Correlation analysis revealed a positive significant association between the age of patients undergoing CABG and the expression of cav-3 (r=0.463; p<0.001; Figure **4**).

DISCUSSION

Neointima formation in the SV aortocoronary grafts' wall followed by its hyperplasia is considered a physiological reaction to high pressure, high stretch forces, and abnormal shear stresses [3, 4]. Unfortunately, it facilitates the development of atheroma that many months and years later may lead to severe stenosis and occlusion of the grafts [2]. Venous SMCs, as the main effector cells of venous graft disease, are not a homogenous cell population and are characterized by variable ability to react to a variety of the released locally biologically active molecules [17]. Moreover not uncommonly, the same types of conduits implanted to the same coronary arteries of a similar degree of stenosis manifested different patency rates and in consequences, late CABG outcomes. There are some suggestions the age of CABG patients and intrinsic venous wall viability may be of importance. However, up to now only a few studies have been focused on morphological and functional cellular variations among SMCs population in the SV segments that could potentially determine long-term outcomes of the SV aortocoronary bypass grafts [18, 19].

This study revealed that expression of caveolins, at least cav-1 and cav-3, in the medial SMCs was associated with the age of CABG subjects. Since caveolins have played a crucial role in membrane trafficking, lipid intracellular metabolism, regulation of calcium homeostasis, lipid and signal transduction in cellular apoptosis as well as proliferation cell signaling [9, 20], the age-dependent variations in their expression may have marked impact on late outcomes after CABG with the use of SV grafts in the age-matched subgroups. Some studies published recently by our team supported the opinion that elderly patients could be particularly benefitted by the SV aortocoronary grafts [16, 18]. Among many published reports about caveolins, there are some that can provide a possible explanation of this aforementioned age-dependent phenomenon. Caveolins, particularly cav-1, have been shown to inhibit activation of the potent growth factors such as PDGF or epidermal growth factor as well as mitogen-activated protein kinase pathways [13, 21]. Thus, it is likely that higher "protective" caveolins expression in the medial SMCs of the SV may be responsible for impairing the process

of the neoitima formation and as a consequence slowing the progression of venous graft disease in the elderly. Moreover, a transformation of the SMCs from contractile to synthetic phenotype observed at the early stages of arterialization of the venous grafts was associated with a loss of caveolae with caveolins [22]. Interestingly, *in vitro* stimulation of the SMCs with human plasma was reported to lead to their proliferation and a decrease in intracellular cav-1 expression [23].

The hypothesis that an age-associated increase in immunostaining for caveolins in the tunica media of the SV grafts must be supported by clinical and angiographic follow up. In the future our research team plans to correlate the preexisting expression of caveolins in the SMCs to a prevalence of adverse coronary events, such as acute coronary syndromes or severe deterioration of CAD, within the late follow up period. The clinical findings will have to be confirmed with coronary angiography. Moreover, we must still wait a few years to draw final conclusions of the clinical significance of stronger caveolins' expression in the elderly because the clinical and angiographic consequences of venous grafts disease (arterialization) are seen 5 to 10 years after primary surgery [24, 25].

CONCLUSIONS

This study revealed significant positive correlations between the expressions of cav-1 and cav-3 in the medial venous SMCs and the age of CABG patients. However, further investigations are necessary to show clinical significance of these age-related variations.

FUNDING

This study was supported by the State Committee for Scientific Research (Grant No. 5958/B/P01/2010/38).

ABBREVIATIONS

BMI = body mass index

CABG = coronary artery bypass grafting

CAD = coronary artery disease

Cav-1 = caveolin-1

Cav-2 = caveolin-2

Cav-3 = caveolin-3

CCAB = conventional coronary artery bypass

LITA = left internal thoracic artery.

OPCAB = off-pump coronary artery bypass grafting

PCI = percutaneous coronary intervention

PDGF = platelet-derived growth factor

PVD = peripheral vascular disease

RA = radial artery

RITA = right internal thoracic artery

SMC = smooth muscle cell

SV = saphenous vein

REFERENCES

[1] Domanski MJ, Borkowf CB, Campeau L, *et al.* Prognostic factors for atherosclerosis progression in saphenous vein grafts: the postcoronary artery bypass graft (Post-CABG) trial. Post-CABG Trial Investigators. J Am Coll Cardiol 2000; 36(6): 1877-83. http://dx.doi.org/10.1016/S0735-1097(00)00973-6

[2] Hassantash SA, Bikdeli B, Kalantarian S, Sadeghian M, Haleh A. Pathophysiology of Aortocoronary Saphenous Vein Bypass Graft Disease. Asian Cardiovasc Thorac Ann 2008; 16(4): 331-6. http://dx.doi.org/10.1177/021849230801600418

[3] Muto A, Model L, Ziegler K, Eghbalieh SD, Dardik A. Mechanisms of vein graft adaptation to the arterial circulation – Insights into the neointimal algorithm and management strategies. Circ J 2010; 74(8): 1501-12. http://dx.doi.org/10.1253/circj.CJ-10-0495

[4] Mitra AK, Gangahar DM, Agrawal DK. Cellular, molecular and immunological mechanisms in the pathophysiology of vein graft intimal hyperplasia. Immunol Cell Biol 2006; 84(2): 115-24. http://dx.doi.org/10.1111/j.1440-1711.2005.01407.x

[5] Rauch BH, Millette E, Kenagy RD, Daum G, Fischer JW, Clowes AW. Syndecan-4 is required for thrombin-induced migration and proliferation in human vascular smooth muscle cells. J Biol Chem 2005; 280(17): 17507-11. http://dx.doi.org/10.1074/jbc.M410848200

[6] Millette E, Rauch BH, Kenagy RD, Daum G, Clowes AW. Platelet-derived growth factor-BB transactivates the fibroblast growth factor receptor to induce proliferation in human smooth muscle cells. Trends Cardiovasc Med 2006; 16(1): 25-8. http://dx.doi.org/10.1016/j.tcm.2005.11.003

[7] Liu P, Ying Y, Anderson RGW. Platelet-derived growth factor activates mitogen-activated protein kinase in isolated caveolae. Proc Natl Acad Sci USA 1997; 94(25): 13666-70. http://dx.doi.org/10.1073/pnas.94.25.13666

[8] Yamamoto M, Toya Y, Jensen RA, Ishikawa Y. Caveolin is an inhibitor of platelet-derived growth factor receptor signaling. Exp Cell Res 1999; 247(2): 380-8. http://dx.doi.org/10.1006/excr.1998.4379

[9] Sowa G. Caveolae, caveolins, cavins, and endothelial cell function: new insights. Front Physiol 2012; 2: 120. http://dx.doi.org/10.3389/fphys.2011.00120

[10] Lahtinen U, Honsho M, Parton RG, Simons K, Verkade P. Involvement of caveolin-2 in caveolar biogenesis in MDCK cells. FEBS Lett 2003; 538(1-3): 85-8. http://dx.doi.org/10.1016/S0014-5793(03)00135-2

[11] Fujimoto T, Kogo H, Nomura R, Une T. Isoforms of caveolin-1 and caveolar structure. J Cell Sci 2000; 113 pt 19: 3509-17.

[12] Millette E, Rauch BH, Defawe O, Kenagy RD, Daum G, Clowes AW. Platelet-derived growth factor-BB-induced human smooth muscle cell proliferation depends on basic FGF release and FGFR-1 activation. Circ Res 2005; 96(2): 172-9. http://dx.doi.org/10.1161/01.RES.0000154595.87608.db

[13] Peterson TE, Guicciardi ME, Gulati R, *et al.* Caveolin-1 can regulate vascular smooth muscle cell fate by switching platelet-derived growth factor signaling from a proliferative to an apoptotic pathway. Arterioscler Thromb Vasc Biol 2003; 23(9): 1521-7. http://dx.doi.org/10.1161/01.ATV.0000081743.35125.05

[14] Epstein AJ, Polsky D, Yang F, Yang L, Groeneveld PW. Coronary Revascularization Trends in the United States, 2001-2008. JAMA 2011; 305(17): 1769-76. http://dx.doi.org/10.1001/jama.2011.551

[15] Ivanov J, Weisel RD, David TE, Naylor CD. Fifteen-year trends in risk severity and operative mortality in elderly patients undergoing coronary artery bypass graft surgery. Circulation 1998; 97(7): 673-80. http://dx.doi.org/10.1161/01.CIR.97.7.673

[16] Perek B, Malinska A, Ostalska-Nowicka D, *et al.* Cytokeratin 8 in venous grafts - a factor of unfavorable long-term prognosis in CABG patients. Cardiol J 2013; 20: (In Press).

[17] Wang Z, Rao PJ, Castresana MR, Newman WH. TNF-α induces proliferation or apoptosis in human saphenous vein smooth muscle cells depending on phenotype. Am J Physiol Heart Circ Physiol 2005; 288(1): 293-301. http://dx.doi.org/10.1152/ajpheart.00165.2004

[18] Perek B, Malinska A, Stefaniak S, *et al.* Predictive factors of late venous aortocoronary graft failure: ultrastructural studies. PLoS One 2013; 8(8): e70628. http://dx.doi.org/10.1371/journal.pone.0070628

[19] Perek B, Malińska A, Nowicki M, Misterski M, Ostalska-Nowicka D, Jemielity M. Histological evaluation of age-related variations in saphenous vein grafts used for coronary artery bypass grafting. Arch Med Sci 2012; 8(6): 1041-7. http://dx.doi.org/10.5114/aoms.2012.32412

[20] Cohen AW, Hnasko R, Schubert W, Lisanti MP. Role of caveolae and caveolins in health and disease. Physiol Rev 2004; 84(4): 1341-79. http://dx.doi.org/10.1152/physrev.00046.2003

[21] Mineo C, James GL, Smart EJ, Anderson RG. Localization of epidermal growth factor-stimulated Ras/Raf-1 interaction to caveolae membrane. J Biol Chem 1996; 271(20): 11930-5. http://dx.doi.org/10.1074/jbc.271.20.11930

[22] Hughes AD, Clunn GF, Refson J, Demoliou-Mason C. Platelet-derived growth factor (PDGF): actions and mechanisms in vascular smooth muscle. Gen Pharmacol 1996; 27(7): 1079-89. http://dx.doi.org/10.1016/S0306-3623(96)00060-2

[23] Peterson TE, Kleppe LS, Caplice NM, Pan S, Mueske CS, Simari RD. The regulation of caveolin expression and localization by serum and heparin in vascular smooth muscle cells. Biochem Biophys Res Commun 1999; 265(3): 722-7. http://dx.doi.org/10.1006/bbrc.1999.1738

[24] Van der Meer J, Hillege HL, van Gilst WH, *et al.* A comparison of internal mammary artery and saphenous vein grafts after coronary artery bypass surgery. No difference in 1-year occlusion rates and clinical outcome. CABADAS Research Group of the Interuniversity Cardiology Institute of The Netherlands. Circulation 1994; 90(5): 2367-74. http://dx.doi.org/10.1161/01.CIR.90.5.2367

[25] Goldman S, Sethi GK, Holman W, *et al.* Radial artery grafts vs saphenous vein grafts in coronary artery bypass surgery: a randomized trial. JAMA 2011; 305(2): 167-74. http://dx.doi.org/10.1001/jama.2010.1976

Received on 27-08-2013 Accepted on 11-10-2013 Published on 30-11-2013

DOI: http://dx.doi.org/10.12970/2311-052X.2013.01.01.3

© 2013 Perek *et al*.; Licensee Synergy Publishers.
This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.